Reference: *Biol. Bull.* **194:** 170–177. (April, 1998)

Paracellular Solute Uptake in the Freshwater Bivalves *Corbicula fluminea* and *Toxolasma texasensis*

HUIYUAN ZHENG AND THOMAS H. DIETZ

Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803

Abstract. Two species of freshwater bivalve were exposed to hyperosmotic solutions of various nonelectrolytes to compare the paracellular permeability of their gill epithelia. In *Corbicula fluminea,* exposure resulted in an elevation of blood solutes that was primarily due to dehydration. After 36 h of exposure, the concentration of Na in the blood decreased precipitously, and the nonelectrolyte accumulated. When lanthanum was added to the solution as a diffusion tracer, its electron-dense precipitate was rarely observed to penetrate the paracellular spaces of the gill epithelial cells in the absence of hyperosmotic stress. In contrast, precipitated lanthanum was commonly observed in the paracellular junctional complexes of the gill in animals that were subjected to hyperosmotic conditions.

When the second species, *Toxolasma texasensis,* was exposed to hyperosmotic solutions of nonelectrolyte, dehydration appeared to be minimal and a seemingly normal concentration of ions was maintained in the blood. This, however, was because of the simultaneous loss of ions and water and a small gain in nonelectrolytes. Longer exposure (12 h or more) produced a precipitous decrease in most blood solutes and an extensive accumulation of nonelectrolyte. More lanthanum precipitate was seen in the paracellular spaces of both control and hyperosmotically stressed *T. texasensis* than in identically treated *C. fluminea.* We conclude that the epithelial junctions found in *C. fluminea* are relatively tight, which probably contributes to the ability of this species to maintain the solute in its body fluid at concentrations higher than are possible in *T. texasensis.*

Introduction

The paracellular pathway is the site of the high electrical conductance of "leaky" epithelia in vertebrates (Frömter and Diamond, 1972). This particular pathway is usually limited by tight junctions, and can operate as a simple "gate" in several epithelia (Diamond, 1977). Paracellular movement of solute has been demonstrated in the epithelia of vertebrate gallbladder, small intestine, and proximal renal tubules (Boulpaep, 1971; Barry *et al.*, 1971; Frömter and Diamond 1972). In molluscs and some other invertebrates, the septate junction performs a function similar to that of the vertebrate tight junction (Lord and DiBona, 1976). When dehydrated, the terrestrial slug *Lehmannia valentiana* displays rapid water uptake and accumulates nonelectrolytes (mannitol and inulin) through paracellular pathways in the foot epithelium (Uglem *et al.*, 1985). In the intestine of the sea slug *Aplysia juliana,* the paracellular pathway constitutes the major conductive route for both Na and Cl (Gerencser, 1982).

In recent studies, the gill epithelia of *Dreissena polymorpha* (zebra mussel) have demonstrated unusually "leaky" characteristics (Dietz *et al.*, 1995; Dietz *et al.*, 1997). When exposed to solutions that were hyperosmotic to their blood, individuals of this freshwater species experienced substantial solute movement through paracellular pathways. Following exposure to hyperosmotic but low ionic strength solutions (100 m*M*) of mannitol, glucose, or sucrose, *D. polymorpha* specimens lost 80%–85% of their blood Na and Cl within 12 h, with concomitant accumulation of the nonelectrolytes from the bathing medium through the paracellular pathway (Dietz *et al.*, 1995; Dietz *et al.*, 1997). Finally, *D. polymorpha* also has a substantial diffusive uptake of Rb from hyposmotic solutions (Wilcox and Dietz, 1995). Paracellular solute movement has not been assessed in any other species of freshwater bivalve.

Corbicula fluminea (Müller) belongs to the family Corbiculidae and entered fresh water during the Pleistocene (Keen and Casey, 1969). *C. fluminea* has been reported to inhabit brackish water up to 5‰ salinity although it is

Received 11 September 1997; accepted 16 January 1998.

considered to be a freshwater species (Filice, 1958; Hayashi, 1956). *C. fluminea* has a higher blood osmolality, a higher rate of ion transport, and a different blood ionic composition than unionid mussels (McCorkle and Dietz, 1980; Dietz 1985; Dietz *et al.*, 1996; Zheng and Dietz, 1998). In contrast to the corbiculids, the unionid bivalves invaded fresh water in the Triassic (Keen and Casey, 1969). The unionid *Toxolasma texasensis* (Lea) and the corbiculid *C. fluminea* may have evolved different epithelial characteristics and, thus, were chosen as bivalve models for a comparative study of paracellular permeability.

In this study, we demonstrate that *C. fluminea* epithelia were relatively non-leaky during a 36-h exposure to a hyperosmotic nonelectrolyte challenge. After the 36-h hyperosmotic challenge the epithelia became selectively leaky, allowing the mussel to gain nonelectrolytes and lose ions, primarily Na. In contrast, *T. texasensis* specimens subjected to similar hyperosmotic conditions had relatively leaky epithelia; they gained nonelectrolytes within 4 h and lost most of the ions from their blood within 12 h.

Materials and Methods

Animals

Corbicula fluminea was collected, under permit, from the Tangipahoa river in Mississippi. Most *Toxolasma (Carunculina) texasensis* and some *C. fluminea* were obtained under permit from ponds in Louisiana. The animals were acclimated to an aerated artificial pondwater (PW) containing (in m*M*) 0.5 NaCl, 0.4 $CaCl_2$, 0.2 $NaHCO_3$, and 0.05 KCl at 22°–25°C for at least 1 week before use.

Blood analyses

Blood was collected anaerobically by cardiac puncture (Fyhn and Costlow, 1975) and centrifuged 1 min at 8000 $\times$ *g*. The supernatant was used for solute determinations. Total solute was measured by freezing point depression on undiluted samples. Concentrations of K and Na were measured by flame photometry, Cl by electrometric titration, and Ca and Mg by atomic absorption spectrophotometry on samples diluted with LaO_3/HCl. The nonspecific phenol/sulfuric acid method was used for rapidly measuring total sugar concentration in large numbers of samples (Montgomery, 1957), and glucose was measured, using a specific glucose kinase procedure (Sigma Chemical), for some samples. Mannitol concentration was determined by HPLC separation using a normal phase APS-hypersil column and a refractive index detector (Gäde, 1991; Dietz *et al.*, 1995). A 5-μl sample of blood in 45 μl of acetonitrile was injected onto the column. Mannitol concentrations calculated from the HPLC chromatography peak area had less than 5% error.

Ion transport

Net ion flux (J_n) was calculated from the change in ion concentration of the bath (Dietz, 1978). The animals were rinsed with deionized water for about 30 min, then each specimen was transferred to a separate 50-ml beaker with appropriate bathing medium. Bath samples were collected at 6-h intervals, and J_n was calculated and expressed as μeq g^{-1} dry tissue h^{-1}.

Hyperosmotic conditions and paracellular permeability

Artificial pondwater containing 100 m*M* mannitol or glucose was used to establish an osmotic gradient without the confounding effect of an elevation of ion concentration in the bath. Each specimen was placed in a separate 50-ml beaker with the indicated bathing medium during an experiment. All animals opened their valves, siphoned the medium, and survived the treatment in hyperosmotic nonelectrolyte solutions. In some experiments, sucrose was used as an osmotic stressor and lanthanum was added to the PW as an electron-dense diffusion tracer to stain the paracellular spaces for electron microscopy (Dietz *et al.*, 1995; 1997). Animals were exposed to CO_2-free PW containing 0.1 m*M* $LaCl_3$ and 100 m*M* sucrose for 2 h or 12 h. Lanthanum carbonate forms an insoluble precipitate at the site of La contact with endogenous HCO_3. The total solute was measured on blood samples collected from the sucrose-treated animals and from controls exposed to the same solution without sucrose. Gills were excised and fixed for 2 h in 2% glutaraldehyde prepared with a phosphate buffer that was isosmotic with the blood of each specimen, and adjusted to pH 7.3 (Gardiner *et al.*, 1991). Tissues were rinsed with a buffer, exposed to 1% osmium tetroxide for 1 h, and dehydrated in an ethanol series. The tissues were infiltrated with 50% LR White resin in ethanol overnight, transferred to 100% LR White resin (2–3 changes), and polymerized at 59°C overnight. Tissues were thin sectioned with an ultramicrotome (50–70 nm), and examined with a JEOL CX-100 transmission electron microscope operating at 40 or 80 keV without further contrasting with heavy metals.

Statistical analyses

Data were expressed as the mean $\pm$ the standard error of the mean, with the number of animals in parentheses. Statistical significance was determined by one-way ANOVA or Student's *t* test. For some data sets in which larger variances were unequal, the data were transformed by the natural log (Ln) for the statistical analysis.

Results

The responses of C. fluminea *to hyperosmotic stress*

Mannitol. Analysis of the blood from animals exposed to 100 m*M* mannitol in pondwater demonstrated that hy-

perosmotic stress induced an elevation in the ion concentrations, increasing the total solute concentration (Table I). After 12 h exposure to 100 m*M* mannitol in PW, osmolality and all ion concentrations in the blood of the animals were significantly elevated, with the exception of K. The average net flux of Na and Cl was unchanged during the initial 12 h of the experiment [control: $J_n^{Na} = 0.30 \pm 0.20$, $J_n^{Cl} = 0.78 \pm 0.16$ ($n = 6$); treated: $J_n^{Na} = 0.13 \pm 0.12$, $J_n^{Cl} = 0.63 \pm 0.09$ ($n = 6$) μeq g^{-1} dry tissue h^{-1}], indicating that the rise in body-fluid solute concentrations was primarily due to dehydration.

A small amount of mannitol was detected in the blood after 12 h of exposure to 100 m*M* mannitol, but after 24 h of exposure the mannitol concentration increased significantly (Table I). These data suggest that the permeability of the epithelium changed after prolonged exposure to the hyperosmotic 100 m*M* mannitol solution. Although the Na concentration in the blood rose further during the 12- to 24-h exposure to hyperosmotic mannitol, there was no difference between the 12-h and 24-h treatment groups for the other ions. These data are consistent with a prolonged osmotic loss of water and a dehydration-induced elevation of ion concentrations in the blood. The penetration of mannitol into the body fluids was likely *via* paracellular routes (see below).

Glucose. Glucose was selected as an alternate nonelectrolyte to induce a hyperosmotic challenge as it allowed more rapid chemical analysis. In most cases the changes of ion concentration in the blood that were induced within 24 h of exposure to hyperosmotic glucose solutions were similar to those produced by mannitol (Table II). At 12 h of exposure to 100 m*M* glucose, the osmolality and the concentrations of all ions except K were significantly elevated in the blood. With the exception of calcium, which continued to rise during the 24 h of exposure, the other ions in the blood showed no further concentration changes

Table I

Comparisons of ion concentrations, total solute, and the accumulation of mannitol in the blood of C. fluminea *over different periods of exposure to 100* mM *mannitol in pondwater*

Solute	Control (n = 24)*	100 m*M* mannitol 12 h (n = 18)	24 h (n = 15)
Na	28.1 ± 0.2[a]	38.0 ± 0.7[b]	43.2 ± 1.0[c]
Cl	23.3 ± 0.5[a]	30.7 ± 0.6[b]	31.1 ± 1.2[b]
Ca	9.0 ± 0.5[a]	14.6 ± 1.5[b]	16.9 ± 1.4[b]
K	0.8 ± 0.0[a]	0.9 ± 0.0[a]	0.8 ± 0.0[a]
Mannitol		1.6 ± 1.1[a]	13.9 ± 3.3[b]
Total	61.5 ± 0.6[a]	85.8 ± 2.2[b]	97.1 ± 2.0[c]

Concentrations are the means ± the standard error, expressed as m*M* or mOsm. In each row, values that are significantly different ($P < 0.05$) are indicated by superscript letters.

* For Na, n = 23.

until 48 h, at which time a critical alteration in epithelial permeability apparently occurred. Small amounts of glucose entered the blood during 12–36 h of exposure to hyperosmotic glucose media. These data are consistent with a dehydration-induced elevation of blood ion concentration over the 36-h treatment.

Although all of the animals lived and appeared to be in normal condition after prolonged hyperosmotic stress, the major ions showed acute concentration decreases concomitant with a rapid rise in blood glucose during 36 to 48 h of exposure (Table II). During this period, 70% of blood Na and 18% to 26% of Cl, Ca, and Mg were lost. Concurrently, the amount of glucose in the blood increased about 8-fold. The concentration of K in the blood was so low that it was below the detection limit (0.05 m*M*) after 48 h of exposure. These data suggest an acute change in permeability of the epithelial tissue following prolonged exposure to hyperosmotic stress.

The blood became isosmotic within a 24-h exposure to the hyperosmotic glucose solution—primarily because of the dehydration-induced elevation of ion concentrations and a rather small amount of glucose entering the blood (Table II). Although most of the ion concentrations in the blood decreased during 36 to 48 h of exposure, the osmolality remained isosmotic to the bathing solution because of the simultaneous accumulation of glucose.

In PW-acclimated animals, the osmolality of *C. fluminea* (about 60–70 mOsm) is higher than that of *T. texasensis* (about 40–50 mOsm). A glucose concentration of 100 m*M* in the bathing medium is more than double the osmolality of the blood of *T. texasensis* (see below). To compare the responses of these two species to an equivalent level of hyperosmotic stress, 140 m*M* glucose was added to PW to challenge *C. fluminea* with an osmolality more than twice the normal level of its blood. After 12 h exposure to glucose in PW, animals in both the 100 m*M* and 140 m*M* glucose treatments had significantly higher blood osmolality [91 ± 1 (n = 5); 96 ± 3 (n = 5) mOsm, respectively] than the PW controls [68 ± 2 (n = 5) mOsm]. However, neither the osmolality nor the major blood ions (with the exception of Ca and Mg; data not shown) in animals from the 100 m*M* treatment group (Na, 47.3 ± 0.4 m*M*; Cl, 34.9 ± 0.5 m*M*) differed significantly ($P > 0.05$) from these in animals exposed to 140 m*M* glucose (Na, 48.4 ± 1.5 m*M*; Cl, 35.7 ± 1.9 m*M*). Likewise, the higher glucose gradient did not significantly change the amount of glucose that entered the blood after 12 h of treatment (3.6 ± 1.8 m*M*; 3.9 ± 0.5 m*M*, respectively).

The responses of T. texasensis *to hyperosmotic stress*

Mannitol. After 24 h exposure to 100 m*M* mannitol in PW, *T. texasensis* experienced a significant decrease in the concentration of all ions in its blood (Table III). Dur-

Table II

Comparisons of ion concentrations, total solute, and the accumulation of glucose in the blood of C. fluminea *over different exposure times to 100* mM *glucose in pondwater*

		100 mM glucose			
Solute	Control	12 h	24 h	36 h	48 h
Na	29.7 ± 0.5[b]	44.2 ± 0.9[c]	45.4 ± 1.0[c]	43.5 ± 0.8[c]	13.2 ± 1.5[a]
Cl	27.6 ± 0.4[a]	35.1 ± 0.5[b]	32.5 ± 1.0[b]	32.1 ± 1.3[b]	26.3 ± 3.0[a]
Ca	8.9 ± 0.7[a]	13.6 ± 1.0[b]	19.8 ± 1.8[c]	14.6 ± 1.0[b]	12.0 ± 1.3[b]
Mg	2.8 ± 0.3[a]	5.2 ± 0.4[b]	5.0 ± 0.4[b]	4.5 ± 0.3[b]	3.3 ± 0.4[a]
K	0.6 ± 0.0[b]	0.6 ± 0.0[b]	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.0 ± 0.0[a]
Glucose	0.7 ± 0.1[a]	4.4 ± 0.8[b]	10.1 ± 2.3[b]	5.5 ± 0.5[b]	44.0 ± 8.0[c]
Total	64.7 ± 1.0[a]	86.6 ± 0.9[b]	96.6 ± 0.9[c]	94.7 ± 1.4[c]	95.0 ± 5.1[c]

Concentrations are the mean plus or minus the standard error of the mean, expressed as mM or mOsm. In each row, values that are significantly different ($P < 0.05$) are indicated by superscript letters; $n = 10$.

ing the hyperosmotic challenge, mannitol accumulated in the blood and accounted for about 71% of the osmolality. In a separate study we monitored the net flux of Na and Cl in *T. texasensis* during a 12-h exposure to 100 mM mannitol in PW. The treated mussels displayed a significant ($P < 0.01$) loss of ions during the study [control: $J_n^{Na} = 0.52 \pm 0.22$, $J_n^{Cl} = 0.04 \pm 0.14$ ($n = 6$); treated: $J_n^{Na} = -2.29 \pm 0.34$, $J_n^{Cl} = -1.82 \pm 0.23$ ($n = 6$) μeq g^{-1} dry tissue h^{-1}].

Glucose. For *T. texasensis* exposed to 100 mM glucose in PW for 24 h, Table IV shows the time course of change in blood solute concentration and the accumulation of glucose. During the 4- to 8-h exposure, animals were able to maintain the concentrations of ions in their blood within the normal range. During this period, there was a small but significant amount of glucose entering the blood. Total solute in the blood was significantly increased due to the slight elevation of the individual ions and the accumulation of glucose from the bathing medium. After exposure of 12 h or longer, there was a precipitous decrease in ion concentrations with concomitant accumulation of glucose in the blood. At the end of a 12-h exposure to 100 mM glucose, the concentration of glucose in the blood was about 70 mM, accounting for 80% to 90% of blood osmolality.

Table III

Comparisons of ion concentrations, total solute, and the accumulation of mannitol in the blood of T. texasensis *before and after 24 h exposure to 100* mM *mannitol in pondwater*

Solute	Control ($n = 9$)	Treated ($n = 6$)
Na	16.9 ± 1.0[b]	2.4 ± 1.2[a]
Cl	9.9 ± 0.8[b]	2.0 ± 0.6[a]
Ca	3.1 ± 0.3[b]	1.8 ± 0.5[a]
K	0.6 ± 0.0[b]	0.2 ± 0.0[a]
Mannitol		61.5 ± 11.0
Total	40.3 ± 2.6[a]	86.8 ± 10.1[b]

Concentrations are the means ± the standard error, expressed as mM or mOsm. In each row, values that are significantly different ($P < 0.05$) are indicated by superscript letters.

Localization of electron-dense lanthanum carbonate

To exclude the possible, but unlikely, existence of an epithelial glucose-transport system in the gills of these animals, we used sucrose instead of glucose as the hyperosmotic osmolyte in lanthanum studies (Dietz *et al.*, 1997). Preliminary studies had demonstrated that both *C. fluminea* and *T. texasensis* behaved the same in sucrose-induced hyperosmotic stress as in glucose treatment (data not shown).

C. fluminea *exposed to lanthanum.* Exposure of *C. fluminea* to 0.1 mM lanthanum in HCO_3-free PW (control animals) for 2 h (not shown) or 12 h resulted either in no deposits of electron-dense lanthanum carbonate, or in only trace amounts in a few locations in the junctional complexes of the gill epithelial cells (Fig. 1A). Only one out of eight control animals was found to contain lanthanum precipitate after either 2 or 12 h of exposure. In contrast, addition of 100 mM sucrose to the bathing medium—all other conditions remaining the same as in the controls—and exposure of four *C. fluminea* resulted in the appearance of lanthanum precipitate in the intercellular spaces between many epithelial cells in all the gill sections examined (Fig. 1B, C). More interestingly, some lanthanum deposits were found to be inside the vesicles or "blister-like" structures inside the epithelial cells of the gill filaments and in the intercellular spaces between the cells (Fig. 1D). The lanthanum-containing blisters were more prominent in the 12-h hyperosmotic treatment than in the 2-h treatment. These blisters were probably formed by the deformation of the lateral membranes of the epithelial cells due to the hyperosmotic-induced dehy-

Table IV

Comparisons of blood ion concentrations, total solute, and the accumulation of glucose in T. texasensis *over different exposure times to 100 m*M *glucose in pondwater*

Solute	Control	100 m*M* glucose 4 h	8 h	12 h	24 h
Na	17.2 ± 0.8^b	19.0 ± 2.2^b	20.1 ± 1.0^b	1.6 ± 0.8^a	0.0 ± 0.0^a
Cl	10.4 ± 0.5^c	11.3 ± 1.4^c	11.5 ± 0.5^c	2.4 ± 0.4^b	0.8 ± 0.2^a
Ca	3.6 ± 0.5^b	5.6 ± 0.9^b	3.9 ± 0.3^b	1.1 ± 0.1^a	1.4 ± 0.1^a
Mg	0.3 ± 0.1^b	0.4 ± 0.0^b	0.2 ± 0.0^b	0.0 ± 0.0^a	0.2 ± 0.0^b
K	0.5 ± 0.0^b	0.6 ± 0.0^b	0.5 ± 0.1^b	0.0 ± 0.0^a	0.0 ± 0.0^a
Glucose	3.5 ± 0.4^a	10.4 ± 5.5^b	10.5 ± 3.2^b	73.2 ± 7.6^c	66.6 ± 8.8^c
Total	40.6 ± 1.8^a	53.0 ± 4.9^b	64.0 ± 3.4^b	80.3 ± 6.0^c	83.4 ± 9.4^c

Concentrations are the mean plus or minus the standard error of the mean, expressed as m*M* or mOsm. In each row, values that are significantly different ($P < 0.05$) are indicated by superscript letters; $n = 5-10$.

dration. This inference was supported by evidence of tissue condensation in the gill filament (Fig. 1D), and by the dehydration-induced elevation of blood solute concentrations observed under hyperosmotic glucose or mannitol challenge (Tables I and II). Deposition of lanthanum in the paracellular spaces of gill epithelial cells was more extensive after 12 h of hyperosmotic stress than after 2 h, indicating a time dependence of the hyperosmotic induction of paracellular leakage.

T. texasensis *exposed to lanthanum.* Animals exposed to HCO_3-free PW containing 0.1 m*M* lanthanum for 2 h (not shown) or 12 h showed only a few locations, in one of the six controls, containing a limited amount of lanthanum deposit in the junctional complexes of the gill epithelial cells (Fig. 1E). When animals were exposed to 0.1 m*M* lanthanum in HCO_3-free PW containing 100 m*M* sucrose for 2 or 12 h, lanthanum was found to penetrate intercellular spaces between many epithelial cells in all the gill sections examined (Fig. 1F, G). Longer exposure to hyperosmotic conditions always resulted in a larger quantity of lanthanum precipitate. In some sections from the animals treated for 12 h, lanthanum precipitate was found within blisters, as observed in *C. fluminea.*

Both the quantity of lanthanum and the number of locations of lanthanum precipitate in the intercellular spaces of *T. texasensis* were greater than in *C. fluminea* after the same treatment time. These data suggest that the paracellular pathways between epithelial cells were more sensitive to hyperosmotic stress in *T. texasensis* than in *C. fluminea.*

Discussion

Under short-term hyperosmotic challenge, the epithelia of *C. fluminea* were relatively "tight" and resistant to the penetration of nonelectrolytes compared to those of another freshwater bivalve, *D. polymorpha* (Dietz *et al.*, 1995, 1997). The initial response of *C. fluminea* to 100 m*M* hyperosmotic stress was an increase, due to dehydration, in the concentration of ions in the blood. During the initial period, *C. fluminea* tolerated a 20% to 50% increase in osmolality and in concentration of the major ions without a significant change in the permeability of its gill epithelia to the nonelectrolytes. The increase in total solute concentration was primarily due to dehydration, as indicated by the lack of any change in the net flux of Na or Cl. However, small but significant amounts of nonelectrolytes were able to diffuse into the blood. That diffusion was probably a result of paracellular leakiness in gill epithelia, which was demonstrated by the presence of lanthanum carbonate precipitates between the cells of the gill filaments after 12 h of exposure to hyperosmotic stress.

Prolonged exposure to hyperosmotic conditions apparently induced a distinct change in epithelial permeability. This is suggested by the acute decrease of ion concentrations and the concomitant rise in glucose observed in the blood of *C. fluminea* after 36 h of exposure to hyperosmotic glucose. The observed changes in solute composition in the blood were most likely due to the increase of paracellular permeability through which glucose or mannitol diffused into the body fluids. It is interesting that the decrease in blood concentration of ions was more pronounced for Na than for any other ion. As much as 70% of the Na, but only 18% to 26% of other ions (Cl, Ca, or Mg), was lost from the blood during 36 to 48 h of exposure to hyperosmotic conditions. The reason for this selectivity was not clear. Perhaps the paracellular pathway is more permeable to monovalent cations and constitutes some proportion of diffusive loss of Na in *C. fluminea,* even under normal conditions. Nevertheless, even after the significant increase of paracellular leakage, the animals were still able to stabilize their blood ion concentrations, except for Na and K, at about control levels. All of the animals survived the treatment regimen, so the

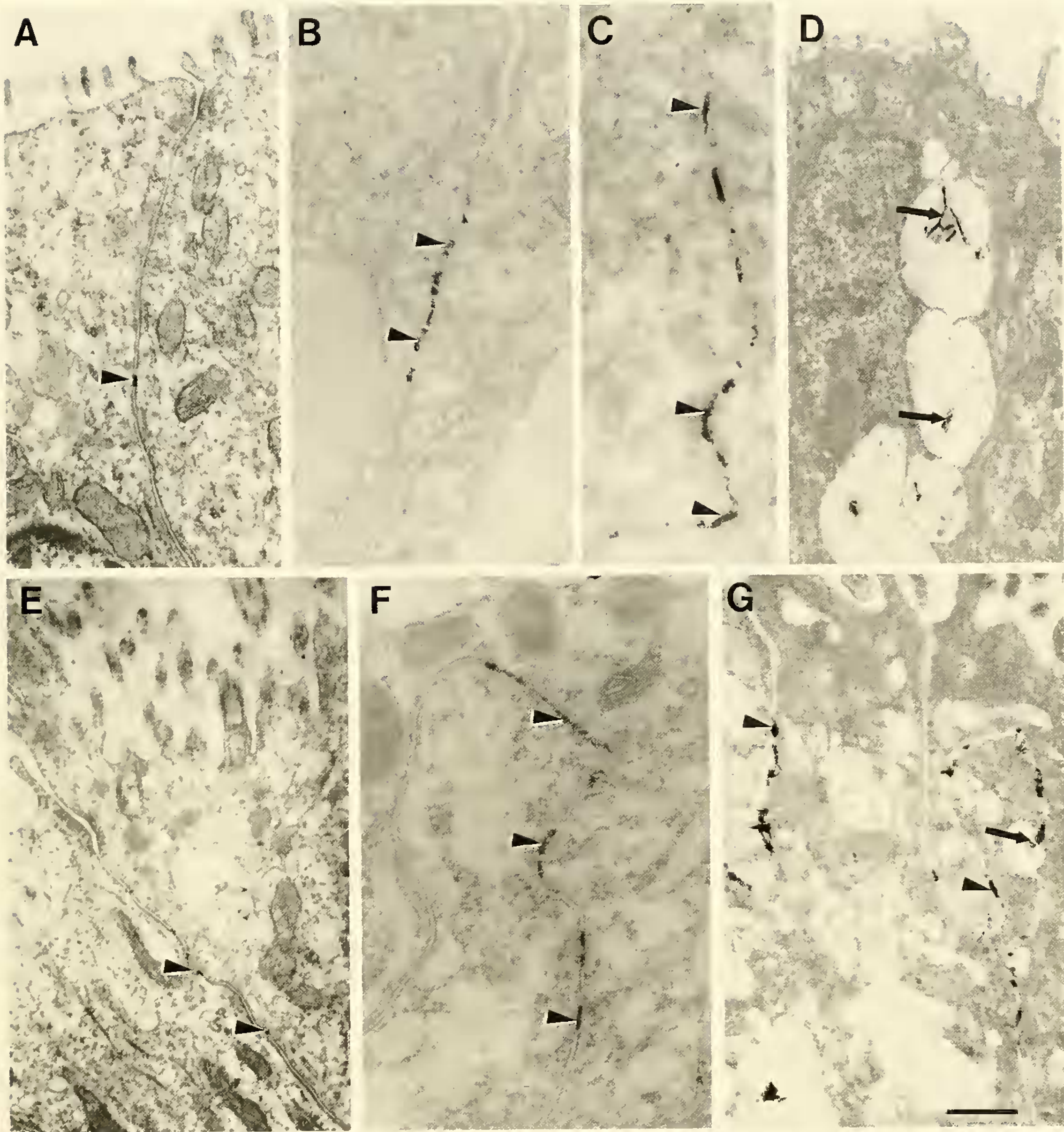

Figure 1. Transmission electron micrographs of gills from *Corbicula fluminea* (A–D) and *Toxolasma texasensis* (E–G) exposed to solutions containing 0.1 m*M* lanthanum. All sections are unstained, and the apical surface is at the top of each micrograph. The calibration bar for all micrographs is at the bottom right corner. (A) Gill epithelium from a *C. fluminea* specimen that was exposed to lanthanum in pondwater for 12 h (control). A small deposit of electron-dense lanthanum precipitate is in the lateral (paracellular) space between cells in the center of the micrograph (arrowhead); bar = 0.46 μm. (B) Gill epithelium from an animal that was exposed to 100 m*M* sucrose in pondwater for 2 h. Small amounts of lanthanum deposits are noticeable in the space between cells (arrowheads); bar = 0.23 μm. (C) Gill epithelium from an animal that was exposed to 100 m*M* sucrose in pondwater for 12 h. Extensive electron-dense lanthanum deposits are present in the space between cells (arrowheads); bar = 0.24 μm. (D) A different region of the same tissue appears more condensed, and a series of lanthanum-containing "blisters" are seen in the lateral membrane region between the epithelial cells (arrows). These blisters may appear as vesicles that are incorporating portions of lateral membranes; bar = 0.89 μm. (E) Gill epithelium from a *T. texasensis* specimen that was exposed to lanthanum in pondwater for 12 h (control). Close to the bottom of the micrograph are lanthanum deposits in the lateral space between cells (arrowheads); bar = 0.49 μm. (F) Gill epithelium from an animal that was exposed to 100 m*M* sucrose in pondwater for 2 h. Electron-dense lanthanum deposits are present in the paracellular space between cells (arrowheads); bar = 0.31 μm. (G) Gill epithelium from an animal that was exposed to 100 m*M* sucrose in pondwater for 12 h. Extensive electron-dense precipitates of lanthanum are found in the paracellular spaces (arrowheads). Lanthanum-containing blisters are present on the right side of the micrograph (arrow); bar = 0.31 μm.

changes were not due to deterioration of the cells or tissues. We have observed that although *Dreissena polymorpha* has the highest paracellular permeability to nonelectrolytes among the bivalves that have been studied, it will survive for more than 2 weeks in 100 m*M* mannitol with little mortality (unpubl. obs.).

The most notable difference between *T. texasensis* and *C. fluminea* is that the former has a lower resistance to long-term hyperosmotic challenge; the difference is probably due to the relative leakiness of the epithelia in the two species. The major change in epithelial permeability occurred after only 8 h of exposure of *T. texasensis* to hyperosmotic glucose, at which time an acute decrease of solutes occurred concomitant with the rapid rise of glucose concentration in the blood. The leakiness was confirmed by the high net loss of Na and Cl from the mussels. As in *C. fluminea,* the increased epithelial permeability was mostly paracellular, as suggested by the increase in lanthanum precipitates between the intercellular spaces after hyperosmotic treatments. Unlike *C. fluminea, T. texasensis* was not able to retain its blood ions after the abrupt change of epithelial permeability. After 12 h of exposure to the hyperosmotic glucose, more than 75% of the ionic solutes were lost from the blood. Furthermore, the loss of the solutes from the blood was relatively nonselective.

Another significant difference is that instead of experiencing an elevation in concentration of the ions in the blood due to dehydration, *T. texasensis* maintained a nearly normal ion concentration for a short exposure (up to 8 h) to a hyperosmotic challenge of glucose. The apparent maintenance of the ion concentration in the blood was coupled with a comparatively low accumulation of glucose in the blood, suggesting that the paracellular permeability was initially relatively low. The slightly increased osmolality was attributed to the small amount of glucose that accumulated in the blood. Despite the dehydrating hyperosmotic conditions, there was no evidence of an elevation in the concentration of ions in the blood of *T. texasensis.* These results suggest that the mussels were losing body fluids so that there was a concomitant loss of solutes and water, as was observed previously in *D. polymorpha* (Dietz *et al.,* 1995). This hypothesis is consistent with the net loss of Na and Cl observed during the hyperosmotic challenge.

The physiological responses of *T. texasensis* to hyperosmotic stress are intermediate, but more similar to those of *D. polymorpha* (Dietz *et al.,* 1995) than of *C. fluminea.* In *D. polymorpha,* approximately 80% to 85% of the Na and Cl in the blood was lost after 12 h of exposure to 100 m*M* mannitol or glucose in pondwater. Within 4 h of exposure to hyperosmotic conditions, total solute was elevated due to the gain of glucose or mannitol in the blood and not to a dehydration-induced elevation of ion concentration. The responses of *T. texasensis* to hyperosmotic challenge are similar to those of *D. polymorpha* in at least two respects. For short-term hyperosmotic exposure, in neither species was there a significant elevation in the concentration of ions in the blood by dehydration. In addition, during 12 h of hyperosmotic challenge both species displayed a substantial increase in the paracellular permeability of the epithelium, allowing the outward loss of electrolytes and the simultaneous inward diffusion of nonelectrolytes from the bathing medium.

In both species, the concentration of potassium in the blood either remained constant as the concentration of other ions increased, or it decreased. These results are consistent with a redistribution of K to the intracellular compartment to provide solute for regulating cellular volume. Similar results were observed in *D. polymorpha* subjected to hyperosmotic challenge (Dietz *et al.,* 1997, 1998). The elevated Ca concentration in the blood of *C. fluminea* likely represents a mobilization of Ca from the shell (Byrne *et al.,* 1989).

The tolerance of freshwater bivalves to an osmotic challenge might reflect their habitats and possibly their origin. *C. fluminea* has been reported to inhabit brackish water up to 5‰ salinity, although it is a freshwater species (Filice, 1958; Hayashi, 1956). Compared to unionid clams, corbiculids are able to maintain a higher osmolality (Dietz, 1979; McCorkle and Dietz, 1980; Zheng and Dietz, 1998). The characteristics of relatively lower paracellular permeability of the epithelia and higher ion transport rates allow *C. fluminea* to maintain the solutes in its blood at a higher concentration than that found in the unionid clam *T. texasensis.* Thus, *C. fluminea* is less sensitive than *T. texasensis* to osmotic stress and more resistant to environmental osmotic challenges.

Acknowledgments

We thank Chengsung Jiang, Janice E. Horohov, Julie Cherry, Diondi Lessard, Paul Bruce, Shawn Wilcox, and Timothy Smith for technical assistance. Harold Silverman provided valuable comments. We thank Ron Bouchard from the M. D. Socolofsky Microscopy Center for his able assistance. This paper was submitted to the Graduate School of Louisiana State University and A&M College in partial fulfillment of the Ph. D. degree by H. Z. The research was supported, in part, by NSF 90-17461, and by Louisiana Sea Grant NOAA 46RG00960 Project R/ZMM-1.

Literature Cited

Barry, P. H., J. M. Diamond, and E. M. Wright. 1971. The mechanism of cation permeation in rabbit gallbladder: dilution potentials and bi-ionic potentials. *J. Membr. Biol.* **4:** 358–394.

Boulpaep, E. 1971. Electrophysiological properties of the proximal tubule: importance of cellular and intercellular transport pathways. Pp. 91–112 in *Electrophysiology of Epithelial Cells,* G. Giebisch and F. K. Schattauer, eds. Springer-Verlag, Stuttgart.

Byrne, R. A., R. F. McMahon, and T. H. Dietz. 1989. The effects of aerial exposure and subsequent reimmersion on hemolymph osmolality, ion composition and ion flux in the freshwater bivalve, *Corbicula fluminea*. *Physiol. Zool.* **62:** 1187–1202.

Diamond, J. M. 1977. The epithelial junction: bridge, gate, and fence. *Physiologist* **20:** 10–18.

Dietz, T. H. 1978. Sodium transport in the freshwater mussel, *Carunculina texasensis* (Lea). *Am. J. Physiol.* **235:** R35–R40.

Dietz, T. H. 1979. Uptake of sodium and chloride by freshwater mussels. *Can. J. Zool.* **57:** 156–160.

Dietz, T. H. 1985. Ionic regulation in freshwater mussels: a brief review. *Am. Malacol. Bull.* **3:** 233–242.

Dietz, T. H., R. A. Byrne, J. W. Lynn, and H. Silverman. 1995. Paracellular solute uptake by the freshwater zebra mussel *Dreissena polymorpha*. *Am. J. Physiol.* **269:** R300–R307.

Dietz, T. H., S. J. Wilcox, R. A. Byrne, J. W. Lynn, and H. Silverman. 1996. Osmotic and ionic regulation of North American zebra mussels (*Dreissena polymorpha*). *Am. Zool.* **36:** 364–372.

Dietz, T. H., S. J. Wilcox, R. A. Byrne, and H. Silverman. 1997. Effects of hyperosmotic challenge on the freshwater bivalve, *Dreissena polymorpha:* Importance of K^+. *Can. J. Zool.* **75:** 697–705.

Dietz, T. H., D. S. Neufeld, H. Silverman, and S. H. Wright. 1998. Cellular volume regulation in freshwater bivalves. *J. Comp. Physiol.* **168:** 87–95.

Filice, F. P. 1958. Invertebrates from the estuarine portion of San Francisco Bay and some factors influencing their distribution. *Wasmann. J. Biol.* **16:** 159–211.

Frömter, E., and J. Diamond. 1972. Route of passive ion permeation in epithelia. *Nat. New Biol.* **235:** 9–13.

Fyhn, H. J., and J. D. Costlow. 1975. Anaerobic sampling of body fluids in bivalve molluscs. *Comp. Biochem. Physiol.* **52A:** 266–268.

Gäde, G. 1991. Hyperglycaemia or hypertrehalosaemia? The effect of insect neuropeptides on haemolymph sugars. *J. Insect Physiol.* **37:** 483–487.

Gardiner, D. B., H. Silverman, and T. H. Dietz. 1991. Musculature associated with the water canals in freshwater mussels and response to monoamines *in vitro*. *Biol. Bull.* **180:** 453–465.

Gerencser, G. A. 1982. Paracellular transport characteristics of *Aplysia juliana* intestine. *Comp. Biochem. Physiol.* **72A:** 721–725.

Hayashi, Y. 1956. On the variation of *Corbicula* due to environmental factors. *Venus* **19:** 54–60.

Keen, A. M., and R. Casey. 1969. Family Corbiculidae, Gray 1847. Pp. 665–669 in *Treatise on Invertebrate Paleontology*, Pt. N, N. R. C. Moore, ed. Geological Society of America, Boulder, Colorado.

Lord, B. A. P., and D. R. DiBona. 1976. Role of the septate junction in the regulation of paracellular transepithelial flow. *J. Cell. Biol.* **71:** 967–972.

McCorkle, S., and T. H. Dietz. 1980. Sodium transport in the freshwater Asiatic clam *Corbicula fluminea*. *Biol. Bull.* **159:** 325–336.

Montgomery, R. 1957. Determination of glycogen. *Arch. Biochem. Biophys.* **67:** 378–386.

Uglem, G. L., D. J. Prior, and S. D. Hess. 1985. Paracellular water uptake and molecular sieving by the foot epithelium of terrestrial slugs. *J. Comp. Physiol. B.* **156:** 285–289.

Wilcox, S. J., and T. H. Dietz. 1995. Potassium transport in the freshwater bivalve *Dreissena polymorpha*. *J. Exp. Biol.* **198:** 861–868.

Zheng, H., and T. H. Dietz. 1998. Ion transport in the freshwater bivalve *Corbicula fluminea*. *Biol. Bull.* **194:** 161–169.